Running Title - Gastric Changes Induced by Ligation

Tital - Ultrastructural Changes of the Parietal Cell in the Ferret Gastric Mucosa Induced by Pylorus-Ligation and Glucocorticoid Administration[1]

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Introduction

Ultrastructural changes in gastric parietal cells have been attributed to stimulatory drugs in the frog (13, 15), cat (21), dog (14, 20), rat (9, 21), mouse (4), and in man (20). Histamine has been utilized most frequently to stimulate parietal cells (12, 14, 16, 18, 21) for cytologic study although other pharmacologic agents have been employed (12, 14). The ulcerogenic capacity of corticoids is well known, but the effects of corticoids on gastric secretion are still debatable (1, 3, 7-9), and the ultrastructural effects of corticoids on the gastric mucosa are generally not known. Hence, it was the purpose of the present investigation to study in part, the gastric mucosal morphology of corticoid-treated, pylorus-ligated ferrets.

The surgical procedure, pylorus-ligation, facilitates the accumulation of gastric secretion for investigative analyses and is widely employed for gastroenterologic studies. However, since the effects of pylorus-ligation on the ultrastructure of the gastric mucosa are unknown, the fine structural alterations in the parietal cell as induced by pylorus-ligation were also evaluated.

Materials and Methods

The present study was undertaken on 40 female, adult ferrets purchased from the Gilman Marshall Ferret Ranch (North Rose, N.Y.), and maintained on a diet of canned dog food (Ken-L-Ration). All animals were pylorus-ligated and were fasted for a period of 48 hrs but received water ad libitum. Five groups consisting of five animals each received the following treatments; subcutaneous injection of 2, 10, or 50 mg/kg body weight of a mixture of hydrocortisone acetate (1.5 parts) and corticosterone acetate (1 part)[1], or 10 or 50 mg/kg body weight of corticosterone acetate. All injections were administered once a day for 3 days. Both preparations were administered in

a corn oil suspension (approximately 1.5 ml per injection). Control animals were injected with pure corn oil, and were either sham-operated or pylorus-ligated. The stomach was removed from each animal during anesthesia and the contents saved for pH, free and combined acid, and volumetric determinations. All animals were sacrificed with an overdose of ether. The stomach was then cut along the greater curvature and small pieces of tissue (1 cm^2) was taken from the lesser curvature at a point midway between the cardiac and pyloric sphincters. Thin slices ($\frac{1}{2}$ mm thick) were cut from the 1 cm^2 piece and fixed for 45 min in 1% OsO_4 buffered to a pH of 7.4 with Veronal acetate containing 7.86% sucrose. Other slices were fixed for $2\frac{1}{2}$ hrs in 5% glutaraldehyde buffered with cacodylate to a pH of 7.4. Tissues fixed in glutaraldehyde were subsequently washed in several changes of cacodylate buffer containing 7% sucrose prior to refixation in 1% OsO_4. All tissues were rapidly dehydrated in a graded series of ethanol and embedded in Maraglas (2). Sections were cut on a Porter-Blum MT2 ultramicrotome, placed on uncoated copper grids, and stained with lead citrate (19) and observed with a Philips 200 electron microscope.

Results

The gastric mucosa of the normal laboratory ferret was previously studied in this laboratory and is reported in a previous paper (18).

The parietal cell of the ferret is apparently very sensitive to pylorus-ligation, while chief cells appear unaffected by the operation. The secretory canaliculi of the parietal cell from pylorus-ligated animals are collapsed (Figs. 1-4) and frequently contain homogenous, moderately electron dense material which probably represents "backflow" of zymogen into the canaliculi from the main lumen of the gland (Figs. 1 & 3). The cytoplasmic content is more extensive and mitochondria are more widely separated than they are in the normal cell (Figs. 1-3). Frequently "ballooning" of the external

mitochondrial membrane occurs, resulting in an electron lucid area on the surface of the organelle (Fig. 1 & 2).

The most striking change following pylorus-ligation is the formation of numerous concentrically arranged smooth membranes within the cytoplasm. These membranes contain small amounts of ground cytoplasm containing several ribosomes. The number of concentric membranes per inclusion varies from one to five or more and the external lamelli may only go part way around the inclusion (Fig. 1-4). Other membranes not organized into concentric swirls may appear as longitudinal profiles within the cytoplasm (Fig. 1, 3, & 4). At high magnification these membranes are comprised of five layers: three electron dense layers on either side of two relatively electron lucid layers (Fig. 4). The total thickness of these membranes is approximately 175 A^{o}.

Parietal cells from pylorus-ligated animals which received corticosterone or a mixture of corticosterone and hydrocortisone showed alterations in the dense bodies, in addition to the above alterations attributable to pylorus-ligation. The dense bodies develop a lighter area within them (Fig. 5) which increases in size until it occupies much of the structure (Fig. 6). The dense bodies per se increase in total diameter, becoming 3 to 4 times as large as those found in the normal cells. Much of their volume is filled, however, with a flocculent electron lucid material (Fig. 6).

The extensive endoplasmic reticulum (ER) seen in normal chief cells is also seen in ligated and/or drug treated animals (Fig. 7 & 8). Small smooth-membraned vesicles were observed pinching off from the rough ER in the area of the Golgi complex (Fig. 8). Similar small vesicles were seen free in the cytoplasm or attached to small zymogen granules in the Golgi zone (Fig. 8). Zymogen granules were also observed being secreted into the glandular lumen. It thus appears that the mechanism for the production and secretion of

zymogen granules was not affected by pylorus-ligation or glucocorticoid administration.

The gastric secretory responses to pylorus-ligation and glucocorticoid administration were evaluated and will be reported in detail elsewhere. Generally, however, the gastric contents of control and treated animals had a volume of approximately 50 ml of fluid at a pH of 2.1, and contained an average of 23.4 milliequivalents of free acid per liter and 46.5 milliequivalents of bound acid per liter. Acute administration of corticoids did not appear to alter the character or trend of acid secretion.

Discussion

While under the influence of acute stress of pylorus-ligation, ferrets develop numerous inclusions within the cytoplasm of the gastric parietal cells. These inclusions consist of concentrically arranged smooth membranes which have not previously been described. A similar cytoplasmic inclusion appears in a micrograph (Fig. 4) in a publication by Sedar and Friedman (14). The tissue was taken from a dog with a gastric fistula, pylorus and cardiac ligations, and under sodium pentobarbital anesthesia. The authors did not mention the inclusion in the text or caption of the publication however.

It is felt that these inclusions are produced in response to the acute stresses of ligation (i.e., surgical stress, anesthesia, increased hydrostatic pressure in the stomach, psychic stress, etc.) are most prominent in the cytoplasm immediately beneath the microvilli, hence they may be derived from the tubular elements described by others (5, 7, 15, 17). Although we have not observed an extensive array of tubular or vascular elements in the normal parietal cell of the ferret (18), it may be that under the conditions of ligation an abnormal amount of agranular membranous material accumulates within the cytoplasm.

In view of these abnormalities, future investigators using the techniques of pylorus-ligation for the study of gastric physiology should take into consideration the possibility of functional alterations due to the operation. Sedar (17) has shown that the tubular elements in the cytoplasm beneath the microvilli of the parietal cell are probably involved in the gastric secretion of hydrochloric acid. If Sedar's explanation of the release of hydrochloric acid is correct it would seem likely that the parietal cells in the present investigation were not actively secreting at the time of sacrifice.

Histamine has been used frequently as a stimulant for gastric production of HCl (14, 16, 17, 20) in ultrastructural studies. Reportedly, tissue has been examined soon after the administration of the drug when the tissue is actively secreting acid. In these cases it has been found that the drug influences the tubular elements in the cytoplasm beneath the microvilli. While corticoids have been shown to have a stimulatory effect on the acid production and various enzymic effects on the intestinal mucosa (9), fine structural studies with animals treated for a short period have not been undertaken. In the only previous electron microscopic study of steroidal effects on the gastric mucosa, it was observed that parietal cells of fasted rats chronically treated with ACTH or prednisone had elaborate canalicular systems (6), in contrast to the collapsed canaliculi observed here. Also, in contrast to the present study, Laumonier and associates (6) observed several alterations in the mucous neck cells of corticoid-treated rats. The discrepancy of these responses suggests that pylorus-ligation induces primarily parietal cell alterations, and that longer term administration of steroids may be required for inducing mucous cell structural alterations.

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Summary

A study of the gastric mucosa of the ferret subjected to pylorus-ligation and glucocorticoid administration has been made. It was found that pylorus-ligation for forty-eight hours causes the formation of numerous concentrically arranged smooth membranes within the cytoplasm of the parietal cell. These inclusions are comprised of one to five or more membranes which contain a small amount of cytoplasm and free ribosomes. Mitochondria are widely separated in comparison to the normal parietal cell and the secretory canals are less extensive and "collapsed."

Glucocorticoid administration brings about an additional alteration in the dense bodies of the parietal cell. An electron lucid area develops within the dense body and increases in size until it fills much of the volume of the organelle. The diameter of the dense bodies increases two- to three-fold and their number is also increased.

Chief cells are apparently unaffected by these treatments.

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Author

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Footnotes

[1] Representative of the ratio of hydrocortisone and corticosterone *in vivo* in the ferret. (Bush; J. Endocrinol. 9: 95:1953).

Fig. 1. A portion of a parietal cell taken from a pylorus-ligated animal. Note the numerous smooth-membraned inclusions (SM). Many of these membranes are arranged in concentric swirls while others are seen in longitudinal profile within the cytoplasm. The secretory canaliculi (SC) are collapsed. Mitochondria (M) are widely separated and some show ballooning of the outer membrane (arrows). The apical portion of a zymogen cell is seen at the lower right and released zymogen is seen within the lumen of the gland (L). Zymogen granule (Z), nucleus (N). x 24,500

Fig. 2. Smooth-membraned inclusions (SM) are seen in the cytoplasm of this parietal cell. A small amount of cytoplasm containing ribosomes is seen with the inclusions. Secretory canaliculi (SC) are collapsed and mitochondria (M) are widely separated. Dense body (DB). x 28,000

Fig. 3. Three dense bodies (DB), which appear to be normal, are seen within the cytoplasm of a parietal cell from a pylorus-ligated animal. The secretory canaliculus (SC) is filled with an electron dense material which surrounds the microvilli. This material is probably zymogen which has "backflowed" into the canaliculus. x 24,500

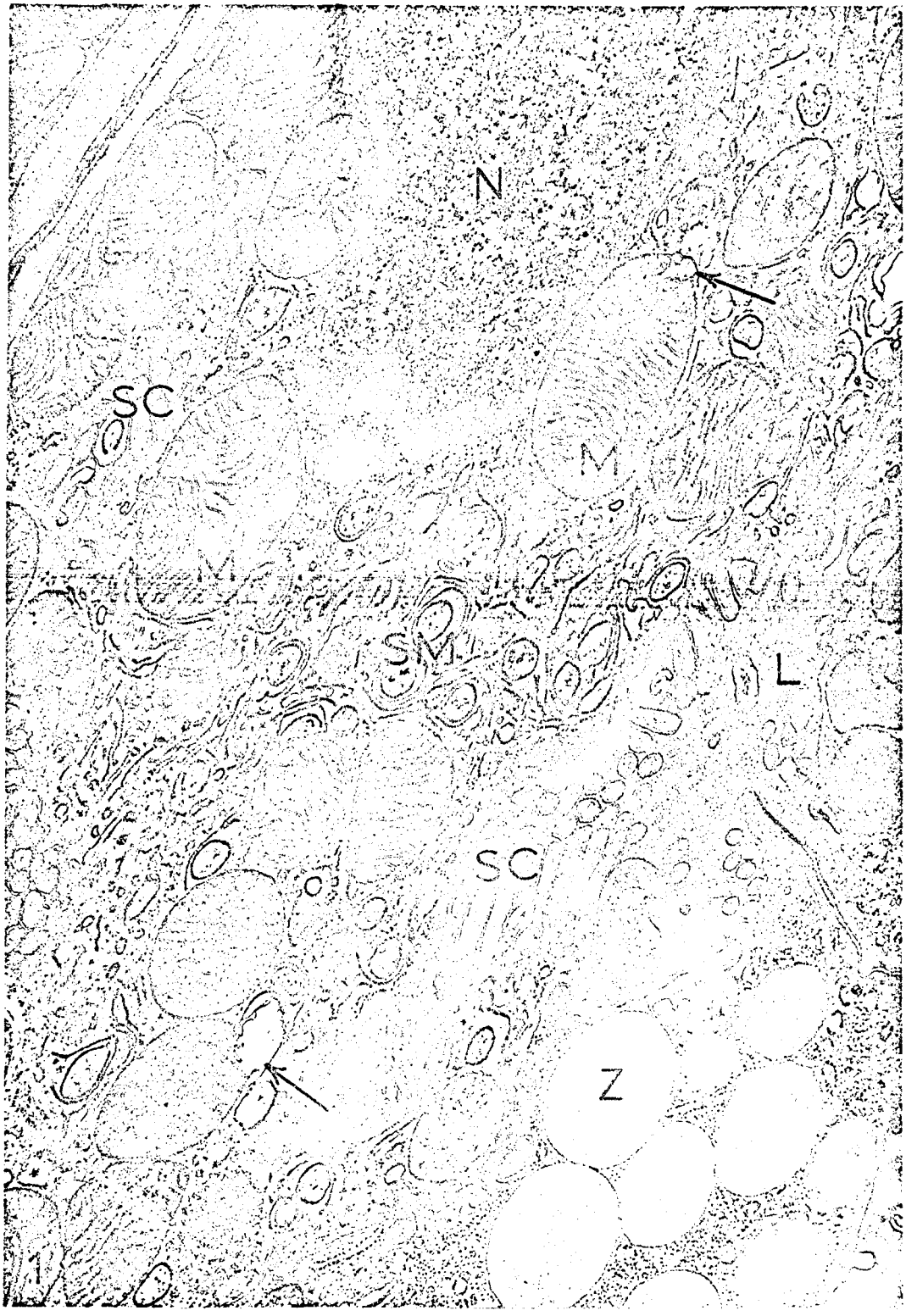
Fig. 4. At higher magnification the smooth-membraned inclusion (SM) can be seen to be comprised of five layers (arrows). Three layers are electron dense and the two between them are more electron lucid. Double-headed arrows indicate fibrils within the microvilli seen in both cross and longitudinal section. Secretory canaliculus (SC). x 60,000

Fig. 5. The tissue in this micrograph was taken from an animal which was pylorus-ligated, and in addition received 50 mg/kg of a 1:1 mixture of corticosterone and hydrocortisone. Note that two of the dense bodies (DB) have spherical light areas within them. Compare this figure with Fig. 6. Smooth-membraned inclusions (SM), secretory canaliculus (SC), mitochondria (M). x 30,000

Fig. 6. A parietal cell taken from an animal treated the same as that in the previous figure. Note the flocculent material which has accumulated in the dense bodies (DB). The diameter of the dense bodies has increased and they are more numerous. Secretory canaliculus (SC), lumen of the gland (L). x 21,000. The inset at the lower left shows a clear relationship between the dense body and the flocculent material. x 21,000.

Fig. 7. Chief cells of experimental animals did not show any major alterations. An extensive endoplasmic reticulum (ER) appears in the basal portion of the gland. Zymogen granules (Z) appear normal. Mitochondria (M) are seen between the cisternae of the endoplasmic reticulum (ER). x 22,000

Fig. 8. The rough endoplasmic reticulum (ER) in the Golgi area (Go) of a zymogen cell from a pylorus-ligated animal is seen in this micrograph. Small smooth-membraned vesicles are seen pinching off from the rough ER in the Golgi area (arrows). Double-headed arrows indicate similar vesicles free in the cytoplasm and one of these vesicles (triple-headed arrow) is seen attached to a small zymogen granule (Z) in the upper left corner. x 51,000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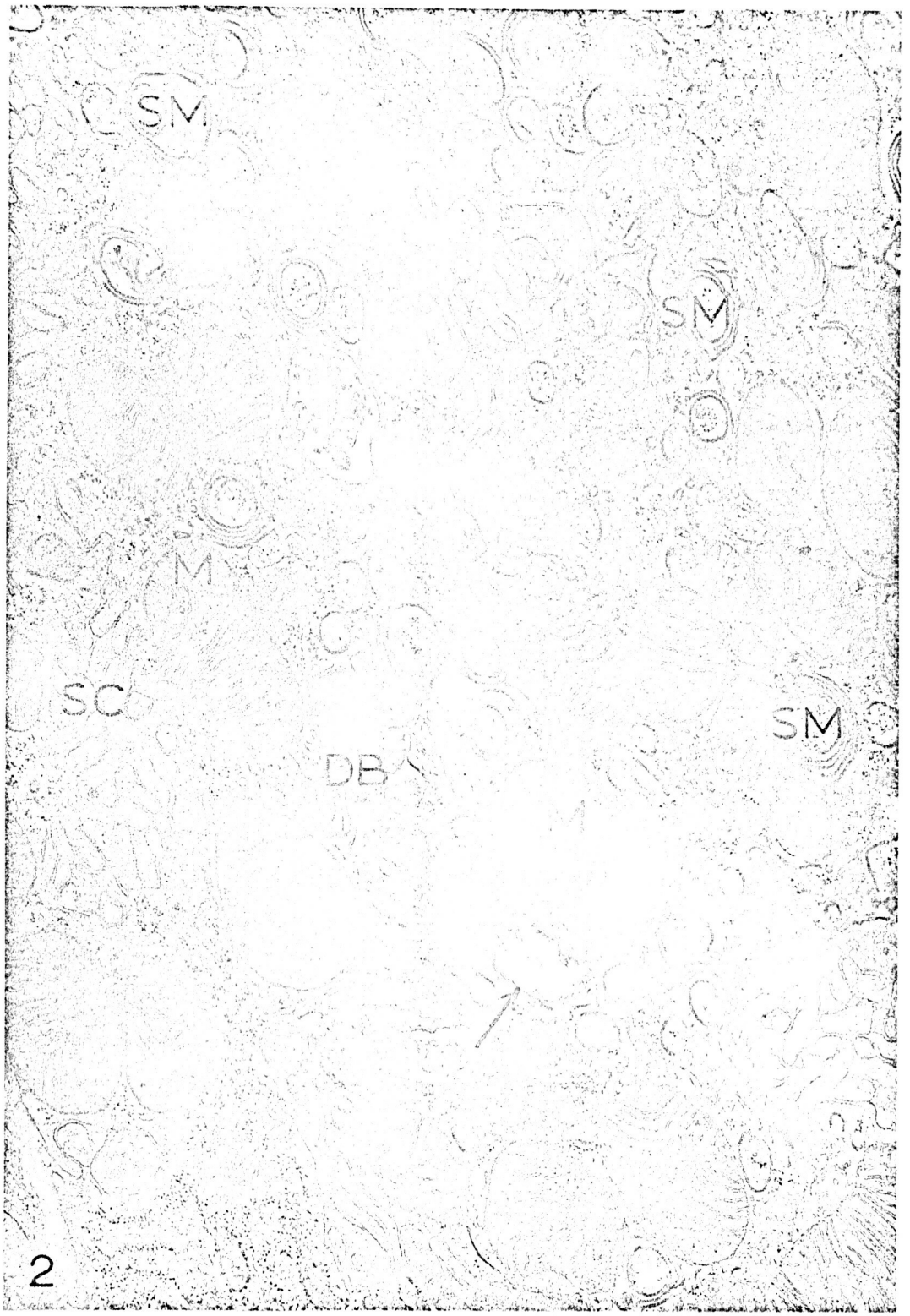
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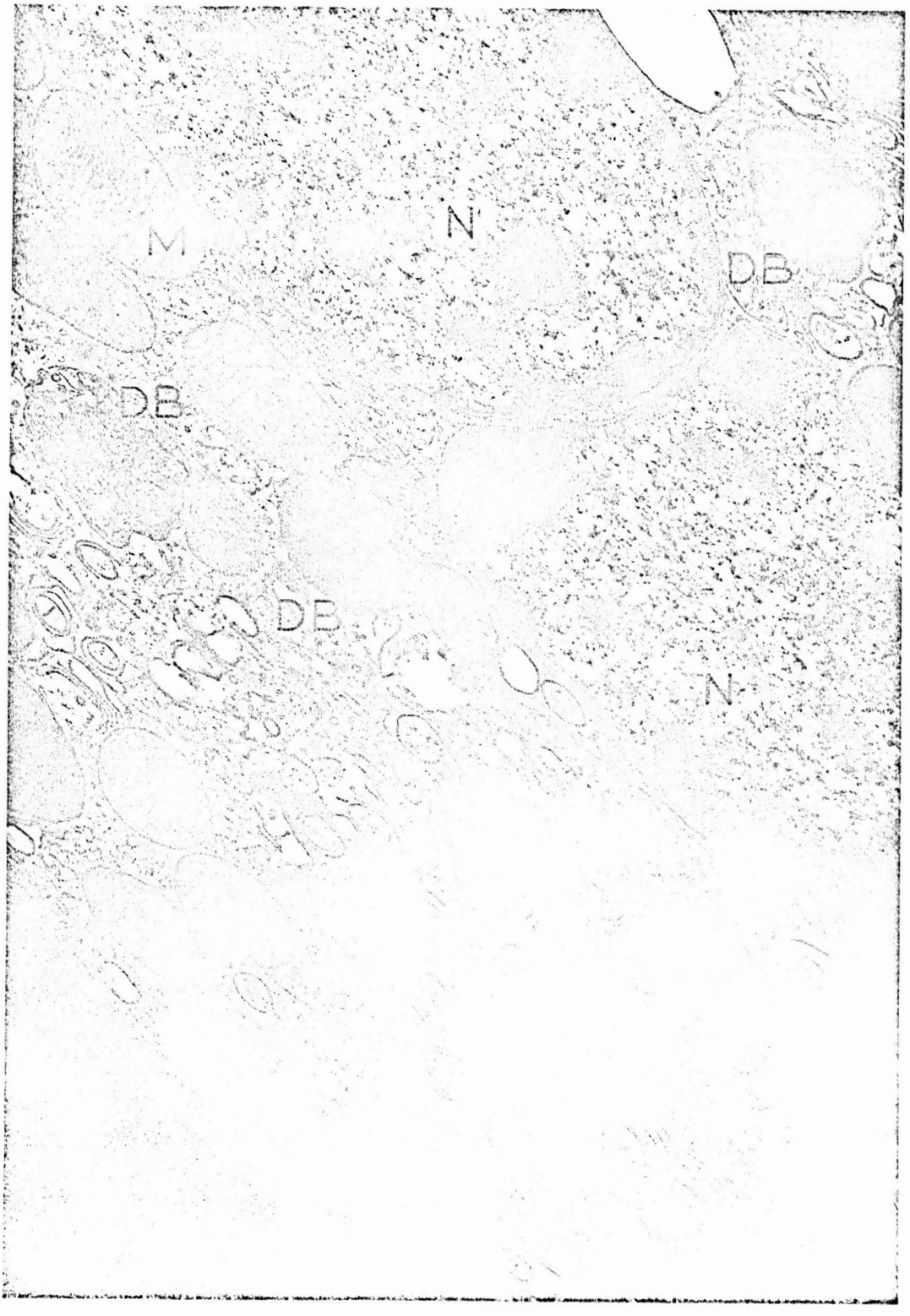
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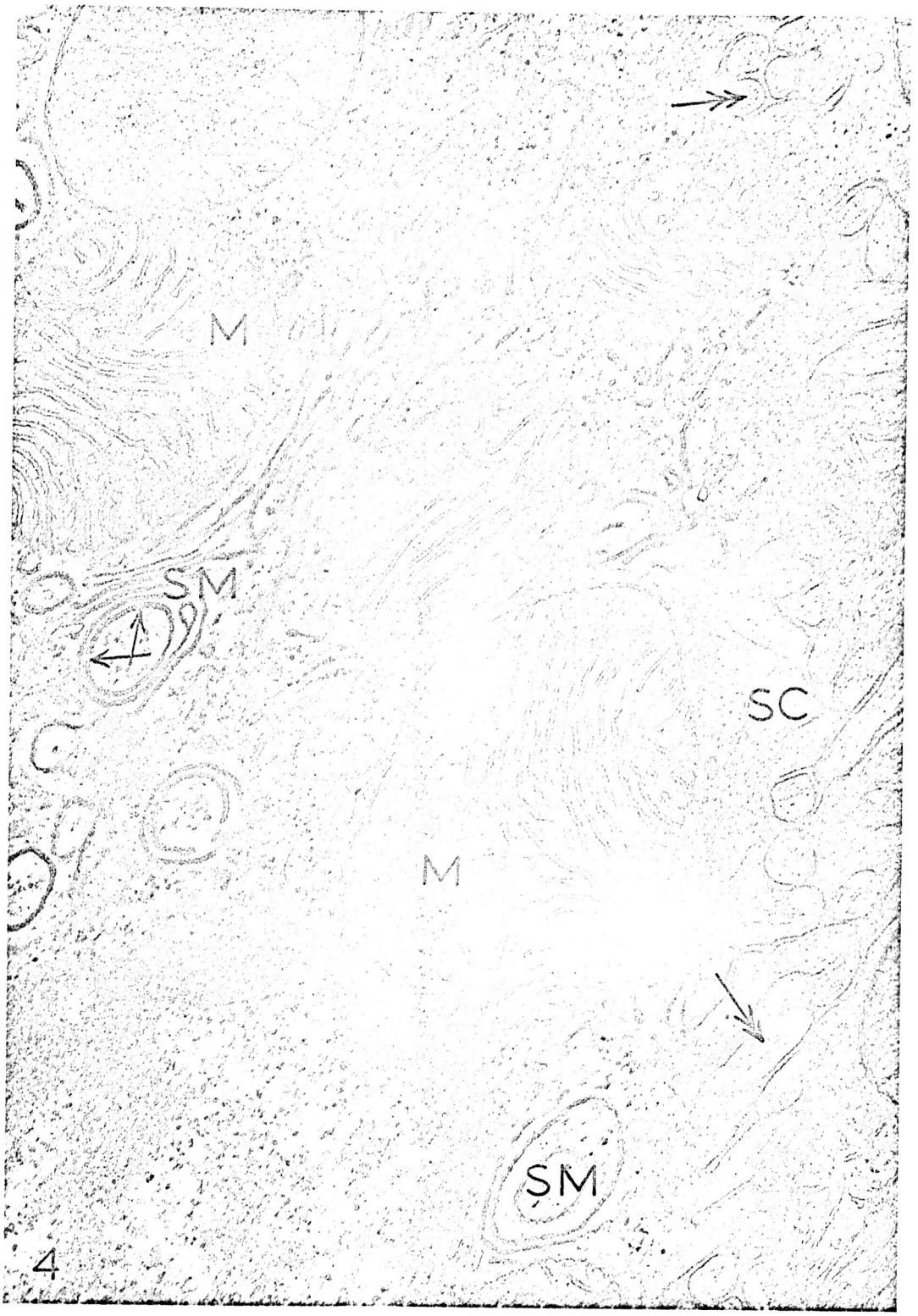

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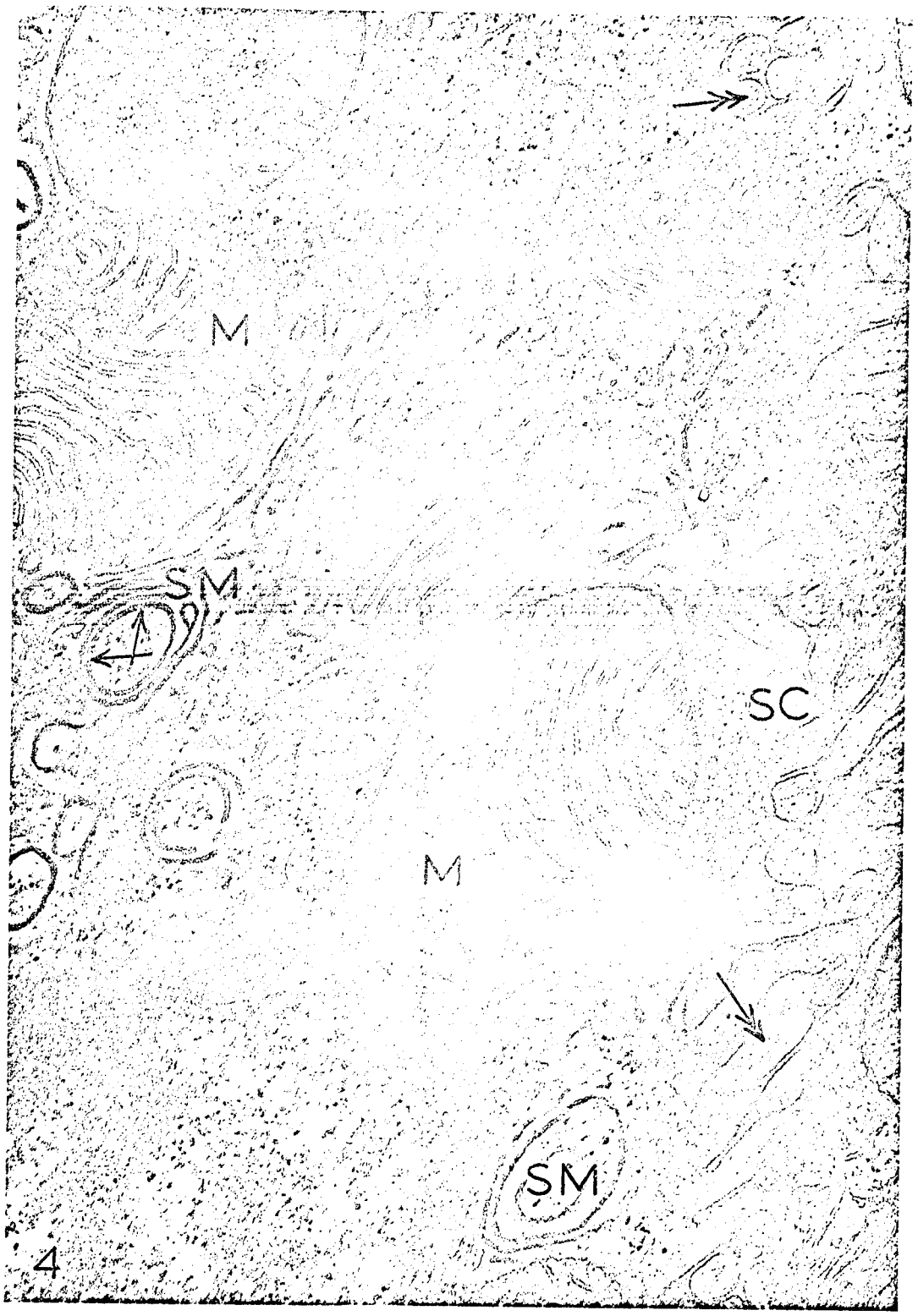
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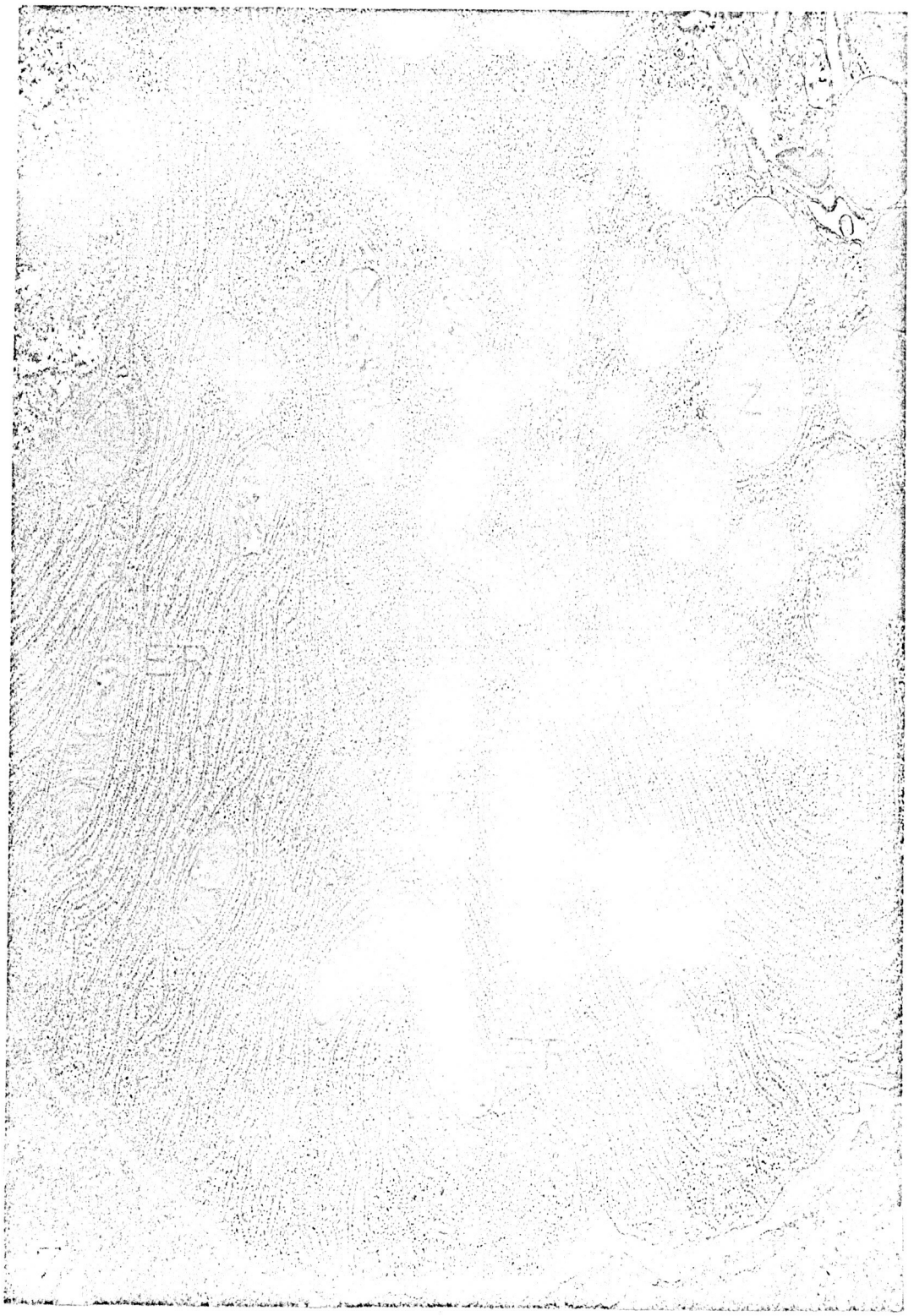

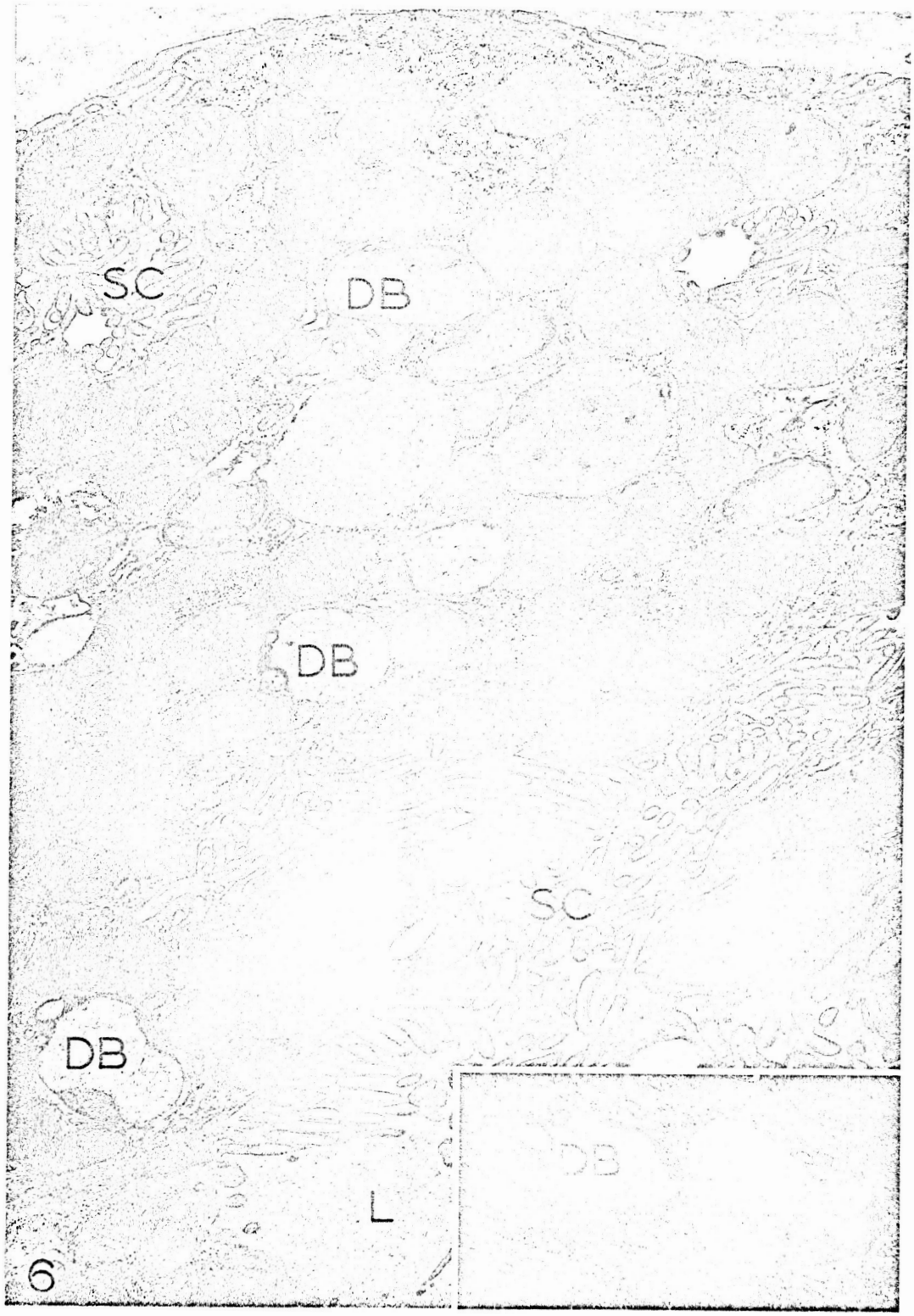
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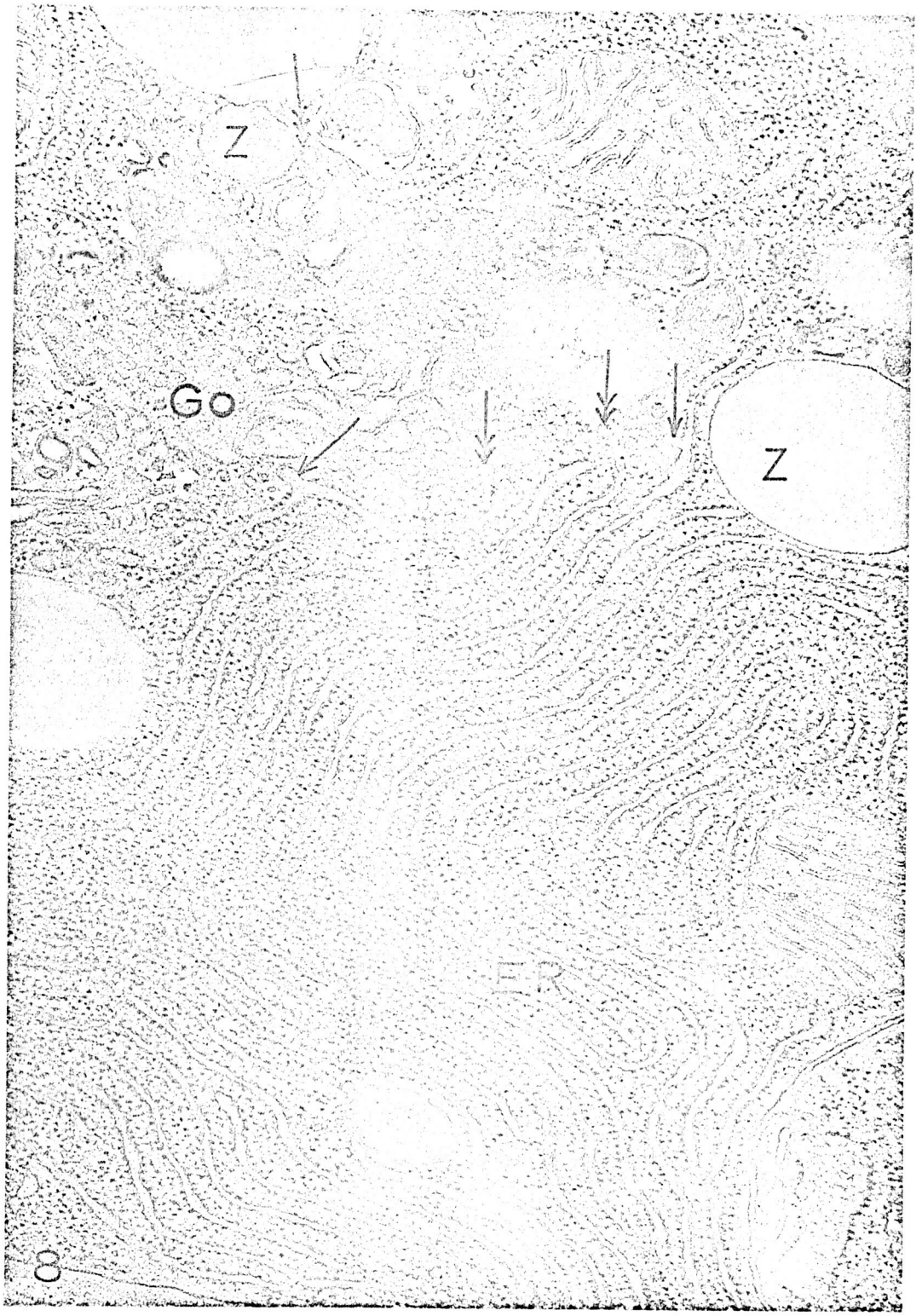
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From Ames
To NASA Representative
Scientific & Technical Information Facility

Subject: Transmittal of Contractor Reports: Stanford Research Institute, Menlo Park, California by C. J. Pfeiffer and R. J. Stephens.

1. The subject reports prepared under Contract NAS 2-3559 have been reviewed at Ames and are recommended for release in STAR as follows:

CR 73138 "Ultrastructural Changes of the Parietal Cell in the Ferret Gastric Mucosa Induced by Pylorus-Ligation and Glucocorticoid Administration"

CR 73139 "Direct Innervation of Capillary Endothelial Cells in the Lamina Propria of the Ferret Stomach"

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N67-38855

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